

Transformations in carbon and nitrogen forms in peat subjected to progressive thermal stress as revealed by analytical pyrolysis

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To study the characteristic N-forms of humic-type materials, samples of sapric peat from Galicia (Northern Spain) were heated at 350 °C for 60, 90, 120, 150 and 180 s, and studied by Curie-point Py-GC/MS, solid-state CPMAS ¹³C-NMR and ¹⁵N-NMR spectroscopies. The NMR analysis of the peat samples in the progressive heating stages showed concentration of heterocyclic N-forms, the maximum structural transition amide-to-heterocyclic forms being observed in samples heated for 120 s (56% heterocyclic N and 34% aromatic C). Under more drastic conditions all N-forms were depleted. Correlation between spectroscopic and pyrolytic data betrayed specific pyrolytic markers for the different N-forms. The intensity of the ¹⁵N NMR amide peak tended to be positively correlated to the yield of indoles, imidazoles and pyrazoles, and negatively correlated to those of benzonitriles and pyrazines. Analytical pyrolysis also showed a progressive enrichment in lipids and alkyl macromolecules with increasing heating intensity, and a decrease in lignin-derived, polysaccharide-derived and N-containing compounds. The relative abundance of non-methoxyphenolic aromatic compounds did not change. The N-compounds in peat samples unheated or heated for 60 to 90 s released methylpyrazole, dimethylpyrroline, methyldiphenylindole and pyrazole, whereas peat samples heated for 120 s mainly yielded methylpyrazine and methyldiphenylindole. Dimethylpyrroline and pyrazine prevailed in samples heated for 150 s, whereas samples heated for 180 s yielded mainly pyrrole. Pyrolysis data presented low possibilities for forecasting the extent of the O-alkyl domain, but reflected quantitatively the transformations of the lignin-like moiety. Both techniques coincide in pointing out the accumulation of a recalcitrant alkyl domain possibly derived from abiotic condensations or inherited lipid biomacromolecules.

Keywords: humification pathways; N-compounds; fire effects; controlled heating

1 Introduction

Despite the potential of current analytical methods to identify the chemical constituents of humic-type materials, to date around 50% of their N consists of unknown structures. Whereas most classical studies postulated that large amount of N compounds in soil organic matter consists of heterocyclic compounds [1], these studies were mainly based on indirect evidence, i.e. the typical fact that only a limited portion of N-structures in soil humic substances yielded aminoacids after 6M HCl hydrolysis [2].

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Further studies based on ^{15}N nuclear magnetic resonance (NMR) evidenced the practical lack of heterocyclic N compounds in most natural soil samples [3], including humus types with large maturity. Experiments based on ^{15}N labelled lignocelulosic biomass demonstrated that thermal processes (i.e. laboratory heatings, wildfires or geothermal processes) on soils and sediments were the most successful in generating non-amide N components producing intense ^{15}N NMR signals in the region of heterocyclic N compounds [4].

Compared to the poor resolution of the broadband NMR spectra of macromolecular substances, analytical pyrolysis combined with gas chromatography and mass spectrometry (Py-GC/MS) is a powerful complementary tool for the structural characterization of humic substances. Due to the high stability of the intramacromolecular links in recalcitrant humic matter, their breakdown requires supplying considerable energy. The disadvantage of this technique consists, in general, of the formation of artifacts and the high yields of cleavage products with very low MW, which are not valid diagnostic fragments for the structural analysis. For this reason, reliable interpretation of the valid “pyrolytic fingerprints” from humic-type substances often requires humic reference material previously characterized, as well as comparisons with the results of alternative, mainly non-destructive techniques.

In the present study, the heterocyclic N-content of a sapric peat was artificially increased by laboratory heating in conditions comparable to those in natural wildfires or controlled burnings [5]. This peat was then analyzed by ^{13}C NMR and ^{15}N NMR for the quantitative assessment of the different N forms (mainly amide or heterocyclic) generated in the course of the heating experiment, and the results were compared with the yields of the different N compounds observed by analytical pyrolysis.

Despite establishing straightforward cause-to-effect relationships between the percentages of different N forms as seen by analytical pyrolysis and ^{15}N NMR is not

intended, it is clear that this experimental approach could be helpful to identify valid surrogate indicators of the degree of diagenetic transformation of amide N in soils and sediments. Also, such an approach could be appropriate to infer some hypotheses about the fire-induced changes in the structural characteristics of the stabilized N domain in humic substances.

2 Materials and methods

2.1 Humic preparations

The peat material was collected from a sapric basement at Buyo (Galicia, northwestern Spain), taken as a composite average sample from the 100 upper cm of the soil. Some general analytical characteristics and spectroscopic ^{13}C - and ^{15}N -NMR data have been reported elsewhere [4, 6].

The peat samples were homogenized by air milling, sieved to 500 μm , and subjected to dry heating according to the experimental conditions previously described [5, 7]. Then, the samples placed in porcelain crucibles were subjected to controlled isothermal heating at 350 $^{\circ}\text{C}$ for progressive periods (60, 90, 120, 150 and 180 s) in an electric furnace (Wosthöff o.H.G, Bochum) operating at atmospheric pressure and under a 625 mL min^{-1} suction flow.

2.2 Solid state CPMAS NMR spectroscopy

The solid state ^{13}C -NMR spectra were obtained with the cross-polarization (CP) and magic angle spinning (MAS) technique with a Bruker DSX 200 spectrometer operating at a frequency of 50.32 MHz, with a zirconium rotor of 7 mm outer diameter with KEL-F caps. The acquisition conditions were those considered to provide reliable quantitative integration values in the different spectral regions [8]. The MAS was performed at 6.8 kHz.

The 90° ¹H-pulse width was of 5.4 μs, and the contact time 1 ms. Between 5,000 and 8,000 scans were accumulated using a pulse delay of 300 ms. Tetramethylsilane (= 0 ppm) was used to calibrate the chemical shift range. Prior to Fourier transformation, a line broadening of 50 Hz was applied. Intensity distribution of the carbon atoms in different structures was determined by integration of signal intensity in various chemical shift regions using the instrument software.

The solid-state ¹⁵N CPMAS NMR spectra were acquired on a Bruker DMX 400 instrument operating at 40.56 MHz and applying a contact time of 1 ms. A 90° pulse width of 5.8 μs, a pulse delay of 150 ms, and a line broadening of 100 and 150 Hz were applied. Between 5 and 7 × 10⁵ scans were accumulated at a magic-angle spinning speed of 5.5 kHz. The chemical shifts were standardised to the nitromethane scale (0 ppm) and adjusted with ¹⁵N-labelled glycine (-347.6 ppm).

2.3 Curie-point pyrolysis/GC-MS

Pyrolysis was carried out in a Curie-point pyrolyzer (Horizon Instruments) attached to a Varian Saturn 2000 GC-MS system. The samples were heated at 510 °C Curie-point temperature for 5 s on ferromagnetic wires. The interface temperature was 250 °C. The liquid CO₂ cryogenic unit was programmed from -30°C (1 min) to 300 °C at 20 °C min⁻¹, and the GC oven was adjusted from 50 °C to 100 °C at 32 °C min⁻¹ and then up to 320 °C at a rate of 6 °C min⁻¹. For the chromatographic analysis a fused-silica capillary column coated with CPSil (film thickness 0.4 μm) and He as carrier gas (25 m × 0.32 mm) were used. Compound identification was based on literature data and further comparisons with mass spectral libraries [9, 10].

In order to present an index that could be related with the extent to which the complexity of the pyrolytic assemblages increased or decreased in terms of the thermal impact, the Shannon diversity index [11, 12] was calculated for the identified compounds.

This index was selected as a conventional measurement of the selective depletion or, conversely, the presence of newly-formed compounds, generated by endothermal reactions which probably concur at the different heating stages.

3 RESULTS AND DISCUSSION

3.1 ^{13}C NMR spectra

Figure 1 shows the CP/MAS ^{13}C and ^{15}N NMR spectra of the peat samples subjected to heating at 350 °C for 0 and 120 s. The major spectral regions, as well as the main signals in the ^{13}C spectra, were interpreted according to standard assignments [13]: 0—46 ppm= alkyl (13= methyl, 21= acetate, 30= polymethylene); 46—110 ppm= O-alkyl (56= methoxyl, 73= carbohydrate-derived); 110—160 ppm= unsaturated aromatic (126= H-substituted, 147= O- or N-substituted); 160—200 ppm= C=O (172= COOH, 198= ketone/aldehyde).

As expected, the ^{13}C -NMR spectra clearly showed that heating led to almost total depletion of the O-containing functional groups, i.e. decarboxylation and dehydration [14]. This was evidenced by the decreased intensity of both carboxyl (173 ppm) and methoxyl (58 ppm) signals in the sample heated for 120 s. Nevertheless, a significant amount of alkyl carbon (30 ppm) presented a thermal stability similar to that of the residual aromatic carbon (128 ppm).

3.2 ^{15}N NMR spectra

Peak assignment for the solid-state ^{15}N NMR spectra (referenced to nitromethane = 0 ppm) were carried out according to Kögel-Knabner [15]: 25 to -25 ppm= nitrate, nitrite and nitro; -25 to -90 ppm= imine, phenazine, pyridine and Schiff-bases; -90 to -145 ppm=

purine and nitrile; -145 to -220 ppm= chlorophyll-N, purine/pyrimidine, imidazole and substituted pyrroles; -220 to -285 ppm= amide/peptide, N-acetyl derivatives of amino sugars, tryptophane, proline, lactams, unsubstituted pyrroles, indoles and carbazoles; -285 to -325 ppm= NH in guanidine, NH₂- and NR₂-groups (N_δ-arginine and N_α-citrulline, N_ε-arginine N_ω-citrulline, urea, nucleic acids and aniline derivatives; -325 to -350 ppm= free amino groups in amino acids and aminosugars; -350 to -375 ppm= NH₄.

As shown in Figure 1, progressive concentration of heterocyclic N-forms (peak centered at -214 ppm) was evident in the ¹⁵N NMR spectra in the course of heating. The transition from amide to heterocyclic forms was observed in samples heated for 120 s, whereas under more drastic conditions (150 to 180 s heating) both amide and heterocyclic N-forms were depleted. In general, the intensity of the ¹⁵NMR amide peak was found to be positively correlated with the yield of indoles, imidazoles and pyrazoles, and negatively correlated with those of benzonitriles and pyrazines.

3.3 Curie-point pyrolysis/GC-MS

The molecular assemblages analyzed by Py-GC/MS suggested a progressive enrichment in the yields of alkyl compounds in the course of heating, with the concomitant decrease in the concentration of methoxyphenols, carbohydrate-derived and N-containing products (Figure 2), whereas the yields of non-methoxyphenolic aromatic compounds remained relatively constant. The decrease in the yields of N-containing compounds with increasing time of thermal treatment was also observed by [16], in an experiment with whole soil subjected to 500 °C for 3 and 9.9 s. Also, these authors observed that increasing the temperature from 300, 500 and then to 700 °C for 3 s did not lead to substantial differences in the identified N-compounds or in their relative ion intensities. Nevertheless, it must be

pointed out that peat and whole soil are expected to show different pyrolytic behaviour since the effect of the mineral matrix in the latter.

It is worthy to point out the fact that the severely heated sample (>150 s at 350° C) showed a dominant, thermal-resistant alkyl domain, as revealed both by ¹³C NMR and Py-GC/MS. Such charred materials, which would be “a priori” ascribed to black-carbon consisting of graphite-like polynuclear structure, in fact seems to consist in a heterogeneous protokerogen-like matrix including resistant polyalkyl or cycloalkyl structures, in addition to a significant domain of “black-nitrogen” structures [17], which after pyrolysis yields mainly pyrazine, methylpyrroline, and pyrrole.

Concerning the nature of the individual N-compounds, the Py-GC/MS results indicated closed dependence on temperature: peat samples unheated or heated at 350 °C for 60 to 90 s consisted mainly of methylpyrazole, dimethylpyrroline, methyldiphenylindole and pyrazole, whereas peat samples heated for 120 s mainly yielded methylpyrazine and methyldiphenylindole (Table 2, Figure 3). Dimethylpyrroline and pyrazine prevailed in samples heated for 150, whereas pyrrole and phthalic acid, 3-nitro (1,2-benzenedicarboxylic acid, 3-nitro) accounted for about the 97% of the N-containing pyrolysis compound in samples heated for 180 s. These changes in the relative yields of the released compounds suggest that at low heating intensities the identified compounds are originated mainly from amino acids (pyrazole, methylpyrazole, methyldiphenylindole) and other still not-elucidated sources, either from plant, microorganisms or soil [18, 19]. With increasing heating time (samples heated for 120 and 150 s) some compounds from hydroxyacids, dipeptides and polypeptides from soil, plant or microbial origin (pyrazine, methylpyrroline) were released [18, 19]. At 180 s of the heating treatment only pyrrole or N-compounds typical from soils (phthalic acid, 3-nitro) have been identified [16, 18, 19,

20]. Schulten et al. [16] indicate that the identification of long-chain alkylnitriles is usually obscured by olefins, which have similar retention times.

The intensity of the ^{15}N NMR amide peak was positively associated to the yield of indoles, imidazoles and pyrazoles, and negatively correlated to benzonitriles and pyrazines. Consequently, these compounds could act as surrogate indicators of the extent of the heterocyclic-N domain in the peat under study, although it is clear that the release of heterocyclic N-pyrolysis compounds do not necessary mean that such structures were present in the original material.

The results of the Shannon biodiversity indices [11, 12] calculated for each group of pyrolysis products is presented in Table 3 and Figure 4. In the case of the non-methoxyphenolic aromatics, carbohydrate-derived compounds and the whole set of identified compounds, the indices showed no linear relationship with the time of heating, with two peaks or maxima at *ca.* 90 and 150 s of heating. The calculated Shannon diversity index for N-compounds showed a similar level in the unheated and the 60–150 s-heated samples, with a maximum at 90 s of heating, whereas in samples heated for 180 s the diversity value dramatically decreased. A similar behaviour was observed for methoxyphenols, where the diversity index increased from 0 to 90 s of heating, then decreasing from 90 to 150 s of heating, whereas no methoxyphenols were detected in the peat sample heated for 180 s. The alkyl compounds (alkanes, fatty acids and olefins) showed very similar trends. The decreased diversity in samples heated from 0 to 150 s could correspond with the decrease observed in the concentration of several compounds, interpreted as a result of active thermoevaporation. At high temperatures (180 s heating) the increased diversity seems to be related to the occurrence of new short-chain alkyl compounds, mainly olefins, resulting from the thermal breakdown of heat-resistant

polymethylene molecules (Figure 5). Also, it was observed a depletion of odd-numbered respect to the even-numbered alkanes and fatty acids.

4 CONCLUSIONS

In general, the quantitative and qualitative changes observed in the compound assemblages of the peat subjected to heating followed a two-stage transformation with progressive heating time. At relatively low heating intensities, the effect of the thermal treatment seemed to be mainly defunctionalization, dealkylation, and generation of newly-formed C and N structures mainly at expenses of carbohydrate and peptides.

This stage was followed by a steady state of selective concentration of the most stable constituents, mainly aromatic but also polymethylene. It was observed that the most frequent N-molecules after 120 s heating at 350° C consisted of heterocyclic and aromatic products. At this stage, the biodiversity indices calculated from the yields of the N-compounds indicated increased complexity of the molecular assemblages with progressive heating. However, at more drastic conditions (in this experiment, after 180 s heating) both pyrolysis and NMR data, together with the calculated Shannon diversity indices, revealed an important reduction both in the relative abundance (number) and diversity of some groups of compounds, mainly methoxyphenols and N-containing ones. This suggests a resilient condensed alkyl domain, together with a black-carbon-like condensed matrix where *O*-alkyl constituents were almost completely depleted or transformed into aromatic structures, and where the N-compounds occur with low number and diversity, indicating concentration of heat-resistant structures yielding mainly pyrazine, methylpyrroline, and pyrrole after pyrolysis.

Finally, the positive correlation of the intensity of the ¹⁵NMR amide peak to the yield of indoles, imidazoles and pyrazoles, and the negative association with benzonitriles

and pyrazines could be used as a surrogate indicator of the extent of the heterocyclic-N domain in the peat under study.

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Table 1. N-containing compounds identified by Curie-point Py-GC/MS in sapric peat samples heated for 0 to 180 s at 350 °C (total abundances calculated from total ion chromatographic area an expressed as percentages of total N-containing compounds)

Compound	0	60	90	120	150	180
Pyrazole	0	0	32	0	0	0
Methylpyrazole	20	21	17	0	0	0
Methylpyrazine	0	0	0	49	0	0
Methylpyrroline	9	0	0	0	0	0
Methylpyrrole I	0	0	0	5	6	0
Pyrrole	0	1	1	1	0	95
Dimethylpyrroline I	18	0	0	0	41	0
Pyrrolidine	0	0	2	0	0	0
Pyridine	0	1	1	3	0	0
Pyrrole methanol	2	0	0	0	0	0
Methylpyrrolidine	13	0	0	0	0	0
Pyrazine	0	0	0	0	19	0
Dimethylpyrazole	0	0	5	0	0	0
Dimethylimidazole	0	3	2	0	0	0
Imidazolemetanol	0	16	9	6	6	0
Trimethylpyrrole	0	0	0	0	4	0
1 <i>H</i> -pyrrole-ethyl-methyl-	0	0	2	0	0	0
Dimethylpyrazole	2	0	0	0	4	0
2,5-Pyrrolidinedione	2	5	3	0	0	0
Hydroxybenzonitrile	0	0	0	1	0	0
Trimethylpyrazole	0	0	4	0	0	0
Methoxypyrazine	7	0	0	0	0	0
Methylpyrimidone	0	8	0	0	0	0
2-(1 <i>H</i>)Pyridinone, 1-methyl	0	0	0	2	0	0
Benzeneacetonitrile	0	0	0	0	4	0

Indolizine	0	0	0	0	0	5
Pyrrolidinone	0	0	1	0	0	0
Indole	0	1	0	0	0	0
Dimethylpyridine2	0	2	3	9	0	0
1 <i>H</i> -pyrrole, 3-ethyl, 2,4-dimethyl-	0	0	0	3	3	0
Trimethylindole	0	0	0	0	3	0
Dimethylindole	6	15	8	4	0	0
Dimethoxypyrimidine	0	0	2	4	0	0
Pyrrolizine-1-methanol, hexahydro-(1 <i>S</i> - <i>cis</i>)	0	2	1	0	0	0
Methyl-diphenyl-indole	20	26	8	14	7	0
1 <i>H</i> -Isoindole-1,3(2 <i>H</i>) dione,2-(1,1'-biphenyl)-4-yl-	0	0	0	0	4	0

FIGURES CAPTIONS

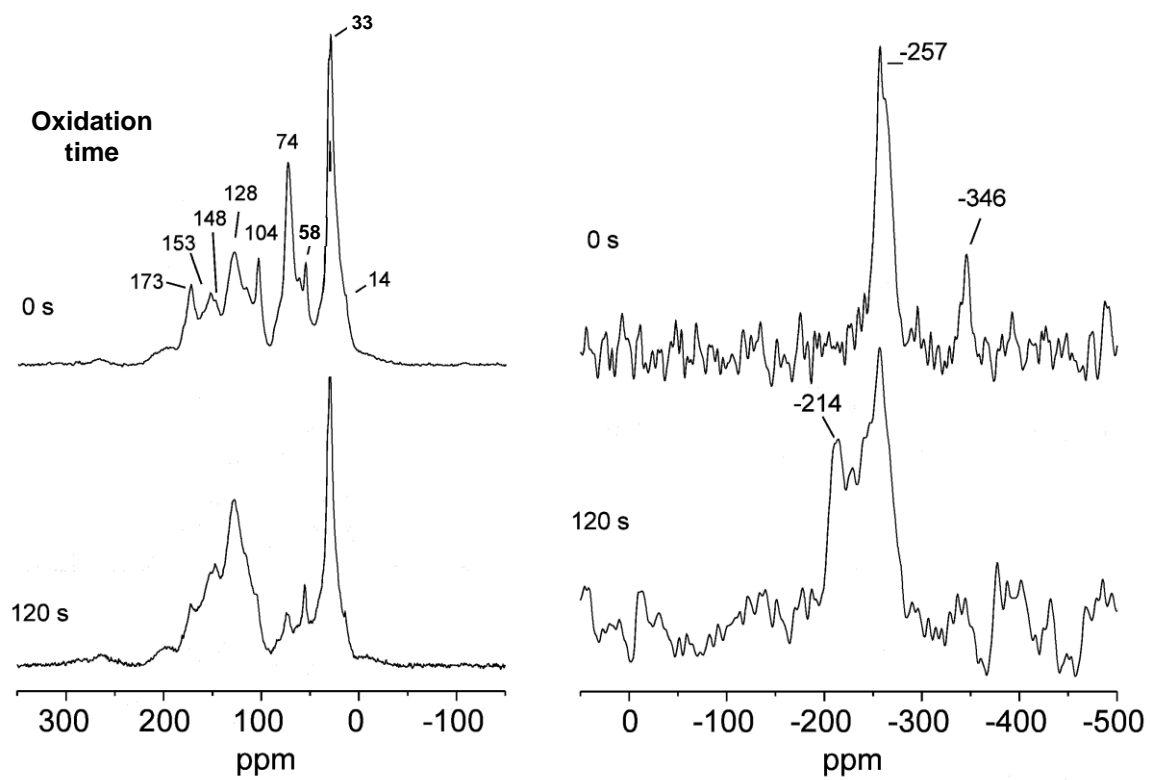
Figure 1. ^{13}C and ^{15}N nuclear magnetic resonance spectra from peat samples subjected to 0 and 120 s of heating at 350 °C.

Figure 2. Kinetics of the release of pyrolytic compounds from peat samples subjected to progressive heating (0 to 180 s) at 350 °C. Semiquantitative data correspond to peak area integration in the gas chromatograms calculated as percentages of the total volatile compounds.

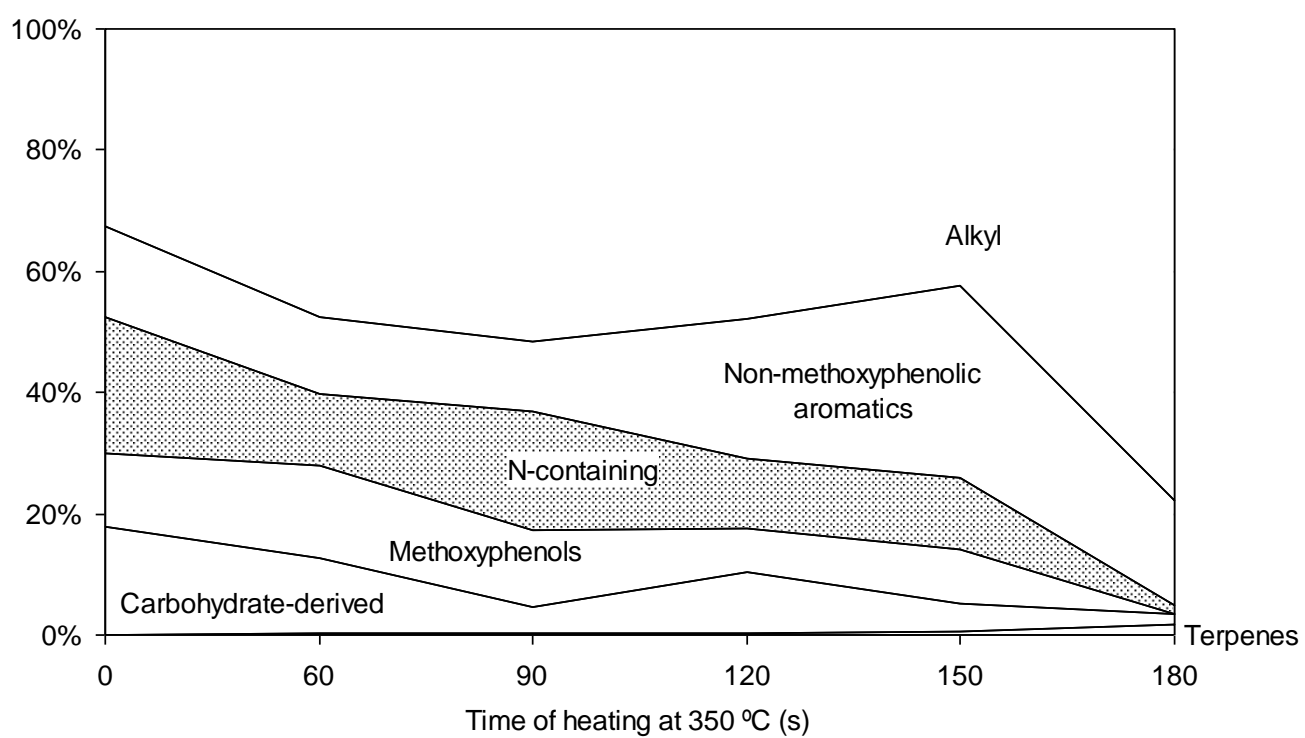
Figure 3. Chromatographic separation range (0 to 10 min) of pyrolysis compounds from peat samples subjected to heating at 350 °C for 0 and 120 s. The structure of the major compounds are shown on the corresponding peaks.

Figure 4. Evolution of possible newly-formed chemical structures in the course of isothermal heating (350 °C) measured as Shannon diversity indices and number of compounds. General: indicates the molecular assemblages of the whole set of identified pyrolytic compounds.

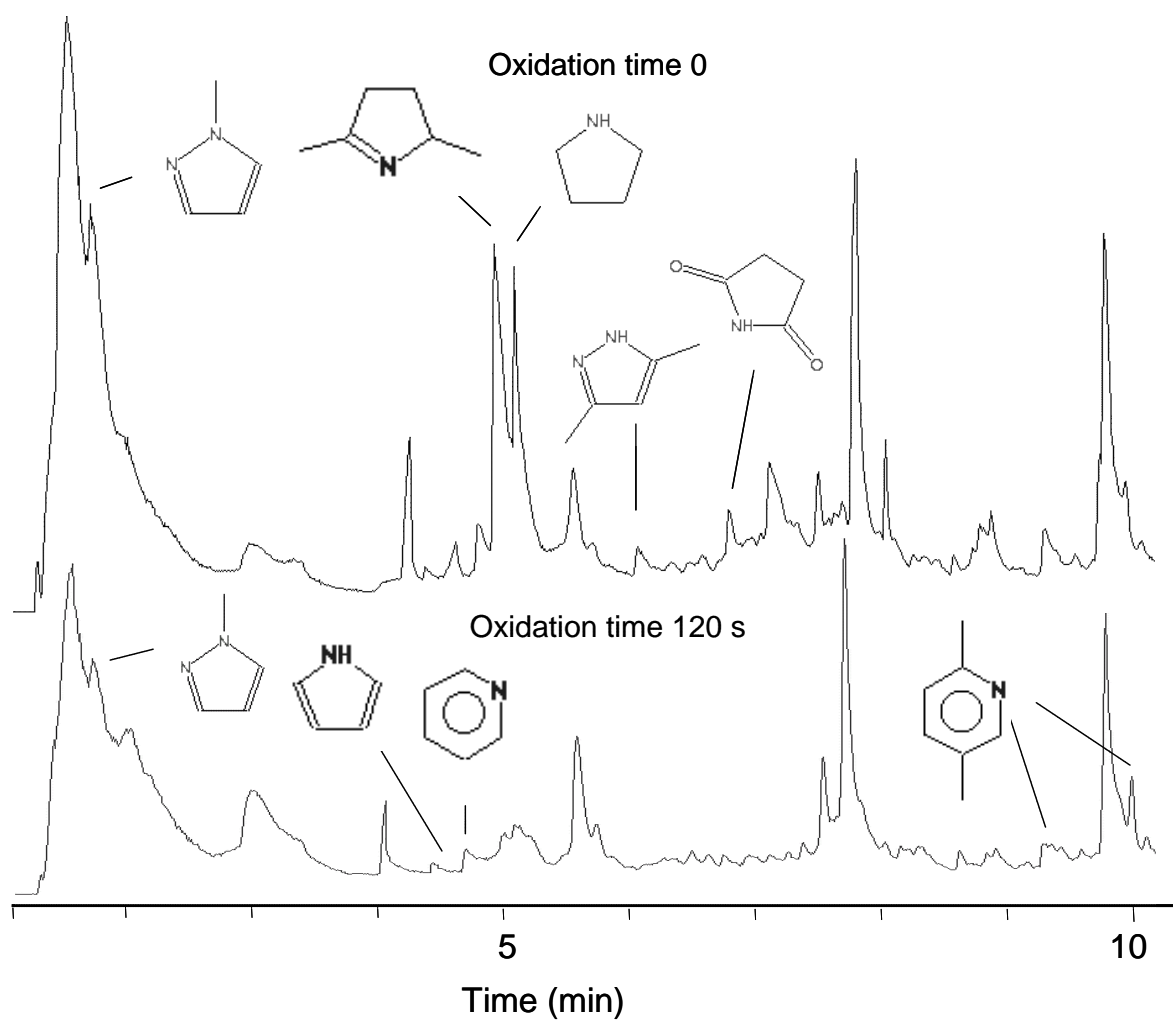
Figure 5. Normalized Shannon diversity indices of the alkyl compounds: alkanes, olefins and fatty acids, in peat samples subjected to heating at 350 °C for 0 and 180 s. The x-axis indicates number of atoms of the carbon chain.



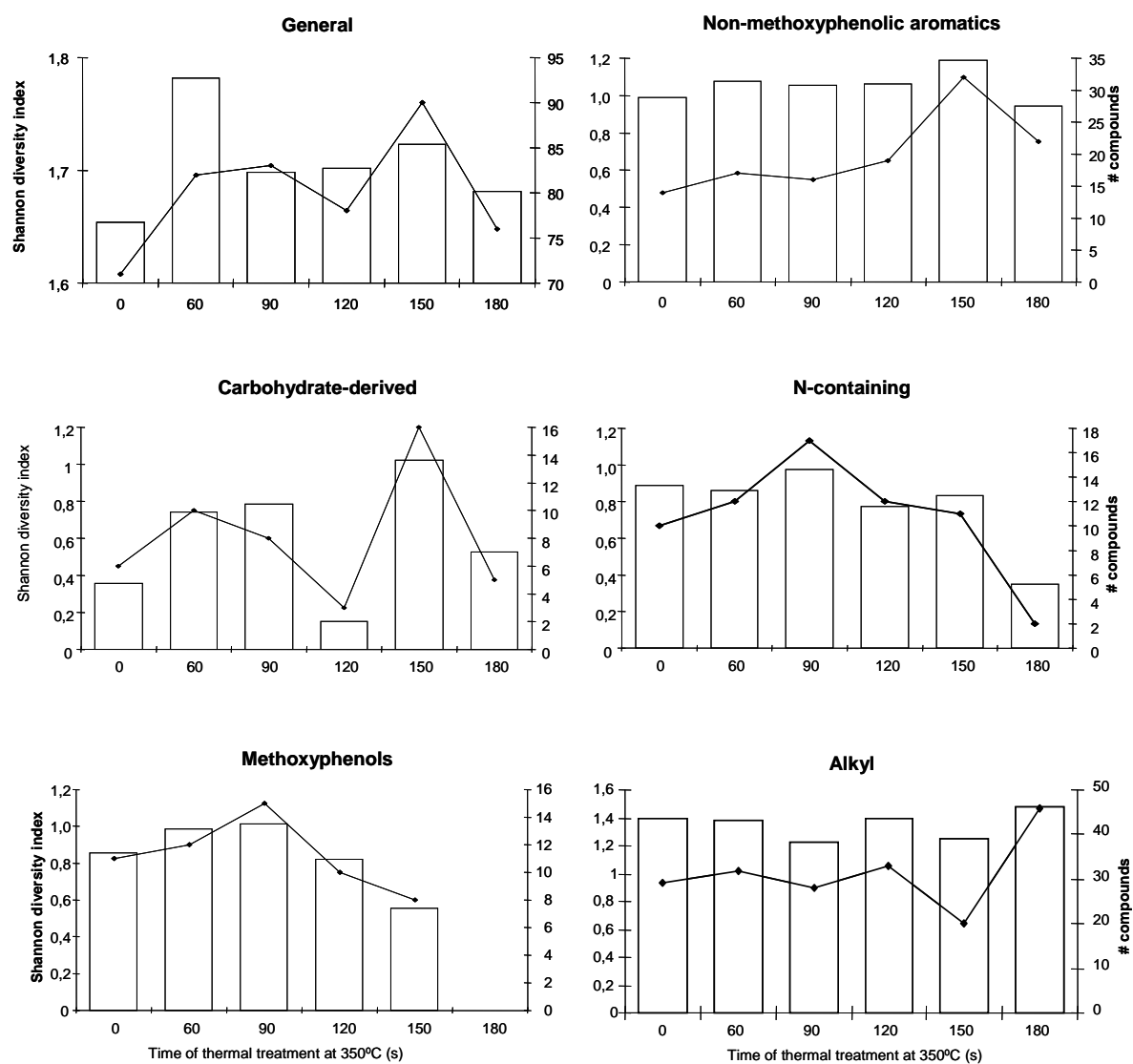
(Figure 1)



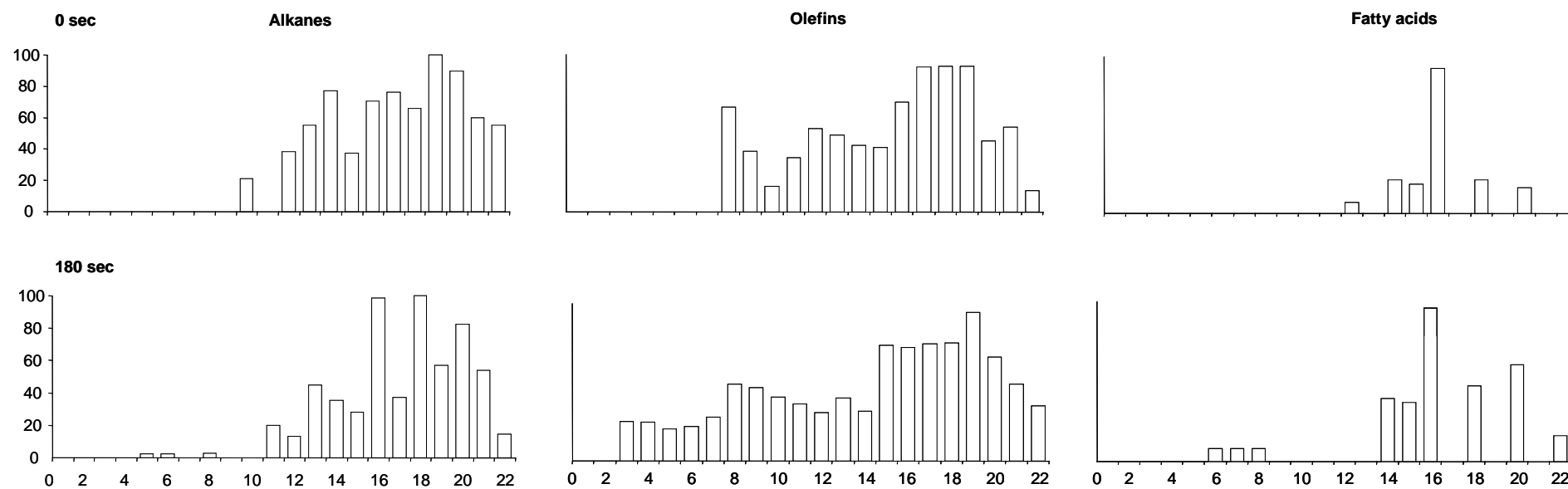
(Figure 2)



(Figure 3)



(Figure 4)



(Figure 5)

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